THE INFLUENCE OF BACTERIA ON DEPILATION OF HIDES BY ENZYMES*

THEONE C. CORDON, A. L. EVERETT, AND W. WINDUS† 1551

Eastern Regional Research Laboratory‡
Philadelphia 18, Pennsylvania

ABSTRACT

Hides and skins as received in a tannery are contaminated with bacteria, and the reduction in salt content after soaking makes them susceptible to degradation. The conditions used for enzyme unhairing also favor the growth of bacteria, a factor ignored by some investigators.

Of a number of disinfectants studied, several were found to control bacterial growth at concentrations which were not harmful to the hair-loosening enzymes.

Although the unhairing solutions were not sterilized, there was no apparent damage to the hide, no putrid odor, and no change in the unhairing action, if the bacterial population was kept under about 100 million per ml.

INTRODUCTION

Although the use of enzymes for loosening the hair on hides and skins has been advocated for at least 50 years (1, 2), little attention has been paid to the possible role of the microorganisms which develop in the liquors. Although disinfectants have been and are used routinely in the commercial application of enzymes for unhairing (2, 3), it seems very likely that many of the results reported were affected by the bacteria present in the unhairing medium. Many workers fail even to mention bacteria, and some (4) have expressed the belief that the results of others were influenced by bacterial contamination.

Numerous workers have studied the "abnormal" bacteriology of hides to seek the causes of various troublesome defects. Others have indicated the large variety of organisms to be found on salted hides and skins. Anderson

*Presented at the 59th general meeting of the Society of American Bacteriologists, St. Louis, Missouri, May 10–14, 1959.

†Biographies of the authors may be found in the February *Journal*.

‡Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(5) was more concerned with the so-called normal flora; he tested a number of proteolytic strains in pure culture but found that only the aerobic spore-formers could digest hide substance, which we (6) have also confirmed. However, he made the interesting observation that most of the strains tested caused complete loosening of the hair (after 3 days at 37°C.). Since any organisms originally present could readily be carried over with the hides, it is important that they be controlled in the enzyme liquors.

Early in our studies of enzyme unhairing (7) a preliminary investigation of some suitable disinfectants was made, and as a result, phenylmercuric acetate was routinely used in our unhairing solutions. Later we included BSM-11, which also contains phenylmercuric acetate. Shuttleworth (8) had recommended zinc or mercuric chloride, among other materials, from a comparative study of soak disinfectants. In a recent study of bactericides for use in sheepskin soaks, Richardson (9) tested a number of materials by a laboratory screening method. Phenylmercuric chloride and several silicofluorides, among others, were found to be effective, while 2-naphthol was not. He noted that bacterial counts ranged from 100 million to 100 billion per ml. in soak waters from packs showing damaged pelts.

When larger-scale tests were started, it was found that if hides were soaked with inadequate amounts of disinfectant, the bacterial population built up to the point where the disinfectants we were using could not control their growth during the enzyme treatment. Under these conditions extremely putrid odors often developed, and extensive hide damage sometimes occurred. Tancous (10) has found a *Clostridium sp.* in salt-cured hides which produces a potent collagenase. This or other collagenase-producing bacteria were no doubt responsible for the hide damage. With proper soaking conditions the disinfectants we used controlled growth satisfactorily.

In an effort to throw light on the problem of inconsistency of hair-loosening results a study was made of the bacterial populations of a large number of enzyme unhairing liquors. The results are reported here.

MATERIALS AND METHODS

Enzymes.—The enzymes used were mostly commercial products. They are listed with their biological sources and suppliers in Table I.

Antiseptics.—The antiseptics, their composition and suppliers, are shown in Table II.

Hides.—The hides were obtained from the M. A. Delph Company, Indianapolis, Indiana. Immediately after flaying they had been fleshed, demanured, and brine-cured. Before use they were washed in a large drum for 1–2 hours and drained.

TABLE I

ENZYMES USED

Enzyme	Source	Supplier
Protease 15 Concentrate	Bacterium	Rohm & Haas Philadelphia, Pa.
Protease 4511-3	Bacterium	Wallerstein Co. New York, N. Y.
Papain	Papaya	Nutritional Biochemicals Cleveland, Ohio
Keratinase	Streptomyces	Institute of Microbiology New Brunswick, N. J.
Viokase	Pancreas	Viobin Corporation Monticello, Ill.
Protease L 56-D L 306, L 305	Bacterium	Pabst Laboratories Milwaukee, Wisconsin
HT Proteolytic, 3, 110 416, 19-20	Bacterium	Takamine Laboratory Clifton, N. J.
HT Concentrate 4903	Bacterium	Takamine Laboratory Clifton, N. J.
Bromelin	Pineapple	Takamine Laboratory Clifton, N. J.

TABLE II

DISINFECTANTS USED

Name	Composition	Supplier
Mersolite-8	Phenylmercuric acetate (PMA) 97.5%	F. W. Berk, Inc. Wood-Ridge, N. J.
BSM-11	10% Phenylmercuric acetate, 50% potassium 2, 4, 6-trichlorophenate, undisclosed solvents	Buckman Laboratories Memphis, Tenn.
Dowicide B	Sodium 2,4,5-trichlorophenate	Dow Chemical Company Midland, Michigan
Sterozol S	Pine oil, cresols orthophenyl phenol, penta-chlorophenol, 2-naphthol	Wallerstein Co. New York, N. Y.
Sodium dithionite	$Na_2S_2O_4$	Eastman Organic Chemicals Rochester, N. Y.
2-Naphthol	2-naphthol	J. T. Baker Chemical Co. Phillipsburg, N. J.

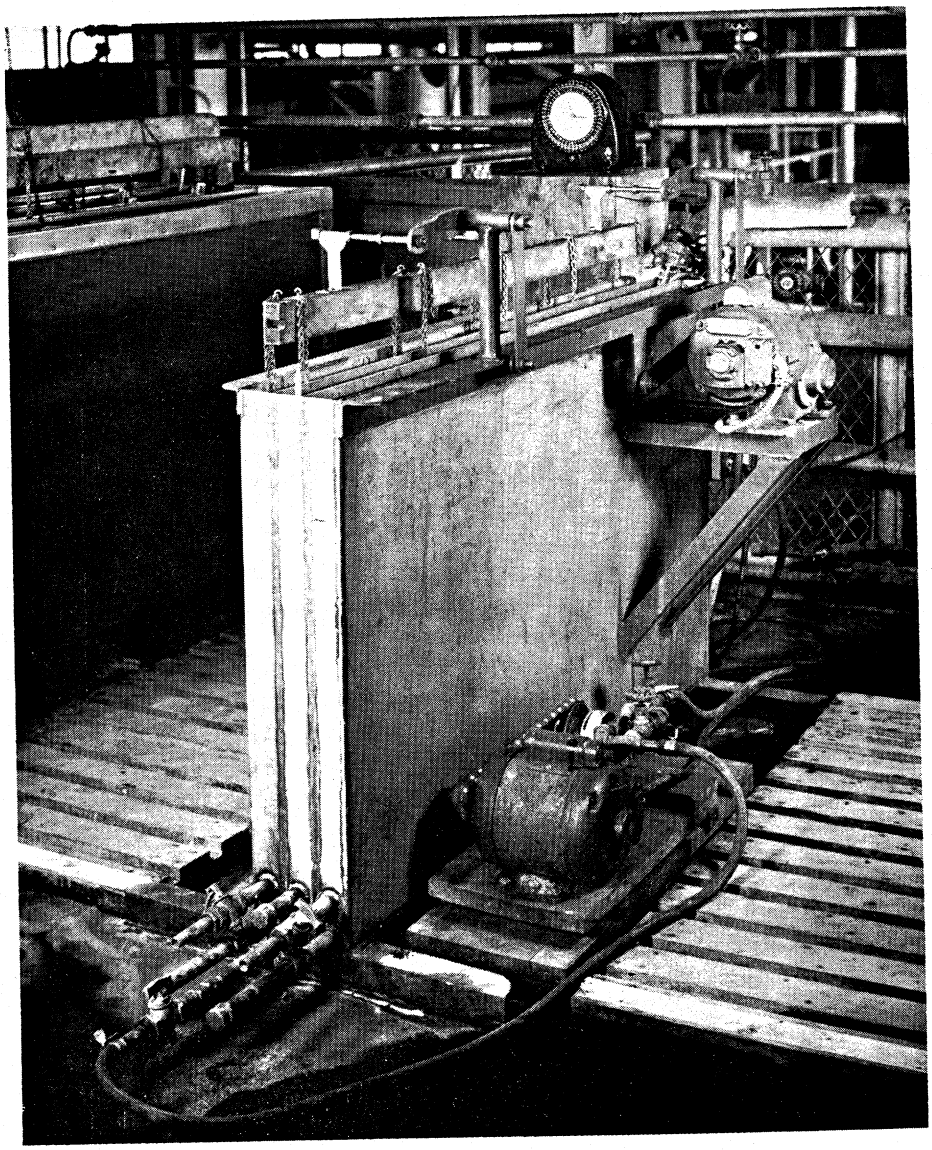

FIGURE 1.—Stainless steel unhairing vat. See text for description.

Unhairing procedure.—The enzyme and additive material under test were dissolved in water and placed in either of the two outer sections of a stainless steel rocker unhairing vat shown in Fig. 1. The center section of the unit contained water and served as a temperature control bath. The water was continuously circulated by pumping out at the top and in at the bottom.

A 1000-watt heater in the bath, connected through a thermoregulator, maintained the temperature at the desired point. Hide pieces consisting of bend and belly or shoulder portions, weighing about 6 to 8 kg., were suspended in the vats. Stainless steel rods were tied to the lower edges of the hides to keep them vertical during rocking. The vats contained 35 l. of solution, making the hide-to-solution ratio about 1 to 5. The hides were rocked at least part of the time they were in the solution, at the rate of 50 cycles per minute with a 4½" stroke. After 20–24 hr. at 30°C. hair looseness was judged by the thumb test and by unhairing with a beam knife. After unhairing, the hides were examined for defects.

Estimation of numbers of bacteria.—Plate counts were made by a standard procedure using Bacto nutrient broth containing 1% NaCl, 0.3% glucose, 0.1% Bacto yeast extract, and 2% Bacto agar. Appropriate dilutions of samples were made in sterile 1% saline. When phenylmercuric acetate was present in the enzyme solution, 0.1% sodium thioglycollate was added to the dilution blanks to counteract this disinfectant carried over in the inoculum.

EXPERIMENTAL

The bacterial population of an enzyme unhairing liquor would be the result of the growth of organisms added with the hide, the water, and the enzyme. Table III shows the numbers of viable bacteria present in some of the enzyme preparations. It is evident that viable bacteria would supply a heavy inoculum to the unhairing liquors. The cells present in the preparations containing a high number of bacteria were found to be mostly in the spore form. Under

TABLE III

NUMBERS OF VIABLE BACTERIA IN ENZYME PREPARATIONS

Enzyme	Plate Count (Air-Dry) millions/g.	Enzyme	Plate Count (Air-Dry) millions/g.
Protease L306	880	Viokase	<0.01
Protease L305*	180	Bromelin	0.09
Protease L 56-D	330	Protease 15 Concentrate	11
HT Proteolytic 110	27		
HT Proteolytic 3	120	Protease 4511-3	0.04
HT Proteolytic 416	217	Papain	<0.01
HT Proteolytic 19-20	16	Keratinase*	4.9
HT Concentrate 4903	50		

*Liquid preparation, count per ml.

the conditions used for unhairing, these spores germinated in a few hours and built up to about 300 million bacteria per ml. within 24 hr. The question naturally arose as to whether these bacteria, which were presumably the same culture which produced the enzyme, would contribute to the depilatory action of the liquor. Several laboratory tests were run with Seitz-filtered HT Proteolytic 3 enzyme. Typical results are reported in Table IV. It is

TABLE IV

THE EFFECT OF REMOVING BACTERIA ON THE HAIR-LOOSENING ACTIVITY OF HT PROTEOLYTIC

Treatment	PMA %	Zero Time	20-hr. Incubation	
		Bacterial Count No/ml	Bacterial Count No/ml	Hair Loosening
None	None	276,000	400,000,000	Very good
None	0.045	106,000	3,870,000*	Very good
Seitz-filtered	0.045	70	3,000,000*	Very good

*Most of these colonies were a *Pseudomonas sp.* which proved, on isolation, to be relatively resistant to PMA but did not affect hair loosening.

clear that filtering out the viable cells did not decrease the unhairing action of the enzyme preparation; nor did allowing them to develop increase it. The fairly high counts which developed in the solutions containing phenylmercuric acetate were due to a *Pseudomonas sp.* originating from the hide. This organism appeared fairly frequently in the unhairing liquors. It was isolated and found to be about six times more resistant to the disinfectants than the remainder of the population. It produced a characteristic aromatic odor but did not exert any hair-loosening action when hide was placed in a whole broth culture. Furthermore, it did not produce any detectable effect when present in the enzyme unhairing solution.

The relative effectiveness of several disinfectants was demonstrated in the following tests: Hides were placed in 35 l. of 0.1% HT Proteolytic 110 solution in the unhairing vat. The disinfectants and their concentrations as percent in solution are shown in the graph (Fig. 2), plotted against the corresponding bacterial count at the end of each test. After 21 to 23 hr. of continuous rocking at 30°C., the hides were removed and unhaired on a beam. The ease of unhairing was rated as very good (VG), good (G), or fair (F) and is shown on the graph. After the loss in volume (about 3 l.) was restored with fresh solution of the same composition, other hide pieces were then treated with the same liquor and evaluated as before. Note that all the counts remained within the 100 million range.

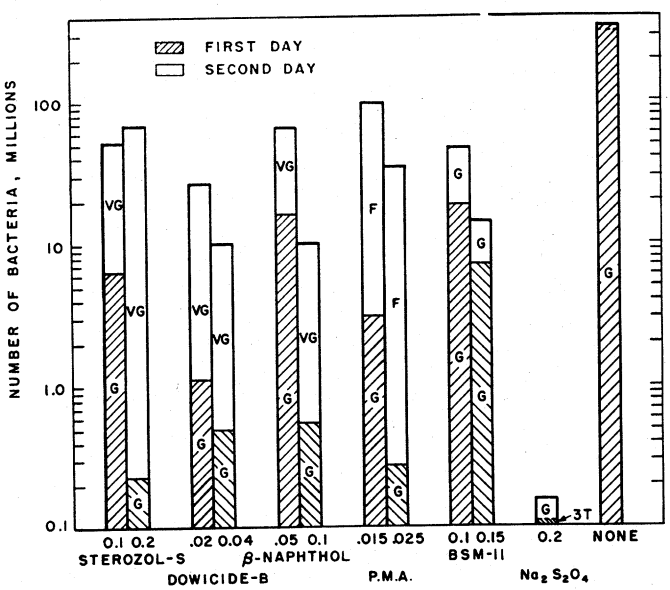

FIGURE 2.—Effectiveness of several disinfectants for controlling bacterial growth in enzyme unhairing solutions. Concentration given is percent in solution. The letters in the bars refer to ease of unhairing. VG = very good, G = good, and F = fair. The cross-hatched sections of the bars show the bacterial count after one use (24 hr.). The open sections show the counts after a second hide had been incubated a further 24 hr.

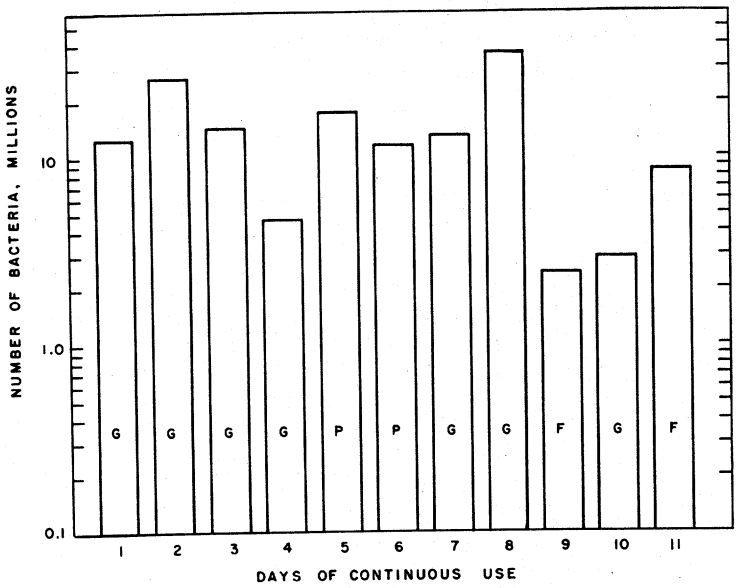

FIGURE 3.—Numbers of bacteria in a continuously used enzyme unhairing solution. The enzyme was HT Proteolytic 0.1%, and the disinfectant BSM-11, 0.1%. The letters in the bars refer to ease of unhairing. G = good, F = fair, and P = poor.

In another experiment an enzyme unhairing solution (0.1% HT Proteolytic 110) was preserved with 0.1% BSM-11 and reused daily for 11 consecutive days. Solution lost by removal of the hide was replaced each day with fresh solution before adding another hide. The results of tests for hair loosening and bacterial numbers are given in Fig. 3. In this series the counts were controlled within a range of 40 million, despite the frequent introduction of more organisms and nutrients.

DISCUSSION OF RESULTS

The results given in Table III show that the bacterial counts of enzyme preparations vary widely. As would be expected, the bacterial enzymes contained the highest numbers of viable cells. They varied between 11 and 880 million per gram of air-dry material and would obviously produce higher initial counts in the unhairing solutions.

The numbers of bacteria developing in such liquors can be quite easily controlled by the use of common inhibitors as shown in Figs. 2 and 3. BSM-11 at 0.1% concentration held the population below 40 million per ml. for 11 days of continuous use. All the disinfectants tested held the count below 100 million per ml. for two days, whereas without disinfectant the count reached 325 million in 24 hr. No evidence of the enhancement of hair-loosening action by bacteria present in the unhairing solutions could be detected. Although some of the results given in Fig. 2 seem to indicate an increase in hair loosening with increasing bacterial numbers, this is probably a coincidence due to hide variation. Data from eight other experiments, with widely varying degrees of control, do not show any correlation of hair loosening with bacterial numbers (Table V).

Under the conditions of these tests, no damage to the hides could be detected even when the bacterial count reached 325 million per ml. of solution, as was the case in the control.

The lack of uniformly good unhairing in the test reported in Fig. 3 is probably due to the low concentration of enzyme used. One-tenth percent of this enzyme is a border-line concentration. Some hides unhair satisfactorily at this level, and some do not. If sufficient enzyme had been used (0.15% to 0.2%), it is believed that the unhairing would have been satisfactory. Our primary objective was to detect any differences due to the bacteria present, rather than to produce consistently good unhairing.

As pointed out earlier, it is well known that if bacteria are allowed to grow unchecked, they may damage hide substance. These results indicate, however, that if reasonable precautions are taken to keep a large population from developing at any step in the processing, no trouble should be experienced with bacteria in a short enzyme unhairing process.

TABLE V

RELATION OF BACTERIAL COUNTS IN ENZYME LIQUORS TO HAIR LOOSENING

Enzyme Liquor		
Times Used	Bacterial Count Thousands/ml	Hair Looseness
1	26	Fair
2	4,400	Fair
1	0.2	Good
2	24,000	Fair
1	55	Good
2	18,000	Good
3	34,000	Good
1	12,600	Good
2	1,410	Good
3	47,000	Good
4	160	Good
1	351,000	Good
2	320,000	Good
1	24.7	Good
2	156	Good
3	115,000	Fair
4	283,000	Fair to poor
1	4,700	Good
2	33,700	Good
3	28,000	Good
1	——	Good
2	7,200	Very good
3	11.9	Very good

It is interesting, and possibly commercially significant, that an enzyme solution could be reused so many times. It appears that if a higher concentration of enzyme had been used in our test, good hair-loosening would have been maintained throughout the entire period.

SUMMARY

If bacteria are allowed to grow unchecked in enzyme unhairing liquors, the hides may be damaged severely. Common disinfectants in moderate amounts will control the growth. If the bacterial numbers are held within about 100 million per ml., the hide substance is not digested. In these tests large numbers of bacteria in the unhairing solution did not increase the hair-loosening action.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Mr. H. J. Willard.

REFERENCES

1. Rohm, Otto. German Patent 268,873 (1910).
2. ———. *Collegium*, 374 (1913). Abstr. *JALCA*, **8,** 408 (1913).
3. Abt, G. *Le Cuir Tech.*, **16,** 514 (1927). Abstr. *JALCA*, **23,** 162 (1928).
4. Gillespie, J. M. *J. Soc. Leather Trades Chem.*, **37,** 344 (1953).
5. Anderson, H. *Ibid.*, **33,** 250 (1949).
6. Everett, A. L., and Cordon, T. C. *JALCA*, **51,** 59 (1956).
7. Cordon, Theone C. *JALCA*, **50,** 270 (1955).
8. Shuttleworth, S. G., and Sebba, J. *JALCA*, **34,** 343 (1939).
9. Richardson, J. H. *Applied Microbiol.*, **6,** 142 (1958).
10. Tancous, Jean J. *JALCA*, **56,** 106 (1961).

Received November 4, 1960.